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RELATIONSHIP BETWEEN AMMONIA EXCRETION RATES AND HEMOLYMPH NITROGENOUS COMPOUNDS OF A EURYHALINE BIVALVE DURING LOW SALINITY ACCLIMATION^{1, 2}

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Marine invertebrates utilize intracellular free amino acids as the solute for cell volume regulation during environmental salinity stress (marine ciliates, Kaneshiro, Holz and Dunham, 1969; cnidarians, Pierce and Minasian, 1974; sipunculids, Virkar, 1966; polychaetes, Clark, 1968; echinoderms, Lange, 1964; molluscs, Lange, 1963; Pierce, 1971b; Lynch and Wood, 1966; Virkar and Webb, 1970; crustaceans, Vincent-Marique and Gilles, 1970; Gerard and Gilles, 1972). In most species taurine, alanine, glycine, proline, aspartic acid and glutamic acid in some combination, comprise the portion of the amino acid pool utilized for volume regulation (Awapara, 1962).

Although the occurrence of this regulatory process is established, explanations of the biochemical mechanism remain in conflict. The most widely published theory is the mechanism proposed by Florkin and Schoffeniels (1965, 1969), based on data from a few species of euryhaline crustaceans. In these species the activity of the ammonia fixing enzyme glutamic acid dehydrogenase (GDH) is modified by external ion concentrations. An increased environmental salinity causes an increased glutamate synthesis and an accompanying decrease in the rate of ammonia excretion. Glutamate then serves as the starting point for several transamination reactions and the amino acid pool size is increased. A decrease in external salinity produces the reverse effect: amino acid pool size is decreased by catabolism to keto-acids and ammonia. Attempts by Florkin, Schoffeniels and various co-workers to apply this mechanism to all phyla are, on ample evidence, unwarranted. For example, amino acids are released unchanged from the cells of isolated bivalve ventricles during

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hypoosmotic volume regulation instead of being degraded to keto-acids and ammonia (Pierce and Greenberg, 1972). Moreover, while little information is available concerning GDH activity in molluscan species, in the few studies conducted, GDH either was not found or had very low activities. Possibly the pathways of amino acid metabolism in molluscs are unique, or perhaps the properties of molluscan GDH are unusual, making detection difficult under the assay conditions used to date (Campbell and Bishop, 1970). However, in view of existing data, cell volume regulation in molluscs during low salinity stress is accomplished by release of intracellular amino acids rather than by a catabolic process. Nonetheless, the whole animal excretion patterns of bivalves (Lum and Hammen, 1964; Emerson, 1969) as well as crustaceans (Jeuniaux and Florkin, 1961; Haberfield, 1971) are ammonotelic.

The combined results of these molluscan and crustacean studies have led us to the hypothesis that during low salinity acclimation by intact bivalves, cell volume is maintained by an efflux of intracellular amino acids into the body fluids. Only after the amino acids have left the intracellular osmotic solute pool does deamination occur, resulting in an increased ammonia excretion rate.

Only one study of changes in nitrogen excretion rate of intact bivalves during salinity stress has been reported (Emerson, 1969) and the results were inconclusive. *Macoma inconspicua* increased ammonia excretion upon transfer from 100% to 50% sea water, while the rate of ammonia loss from *Mya arenaria* remained unchanged. Moreover, no measurements of blood ammonia or amino acid concentration have been correlated with ammonia excretion rates of bivalves acclimating to different salinities. Thus, the whole animal nitrogen excretion response during salinity stress has not yet been established in molluscs.

We have investigated the effect of lowered external salinity on whole animal ammonia excretion rates and on concentrations of some hemolymph nitrogenous compounds of a euryhaline bivalve, *Modiolus demissus demissus* Dillwyn, both during and following acclimation. The results show that following a decrease in external salinity, an increase in hemolymph amino acid concentrations occurs which coincides with a decrease in the tissue free amino acid pool. As acclimation proceeds, blood amino acid concentrations level off in company with an increase in both blood ammonia concentration and external ammonia release. Finally, hemolymph amino acid concentrations return toward control levels, followed by a decrease in hemolymph ammonia concentration and external ammonia excretion rate.

MATERIALS AND METHODS

Animals

Modiolus demissus were collected from a salt marsh on the Chincoteague Bay side of Assateague Island, Maryland. They were kept in aquaria containing aerated artificial sea water (Instant Ocean: salinity = 35‰ = 1020 mOsm/kg H₂O = full strength sea water, hereafter) in the laboratory (22° ± 1° C) at College Park, Maryland. All animals were acclimated to full strength sea water for at least three weeks before being used experimentally.

Preparation of animals

Twenty-four hours before the start of each experiment, animals were removed from the main aquarium and the valves were scrubbed thoroughly with a brush

to remove fouling organisms. The mussels were then propped open with pieces of glass tubing inserted between the ventral valve margins. This procedure prevented the animals from closing and thus ensured constant exposure of the soft parts of the animals to the experimental sea water conditions. The propped-open animals were kept together in a container of aerated Millipore filtered (0.45μ) full strength sea water for 24 hours.

Ammonia excretion rates during acclimation

Following the preparation described above, propped-open *M. demissus* were placed individually into beakers containing 700 ml of either filtered full strength sea water (control) or filtered 50‰ sea water (510 mOsm/kg H_2O) (experimental) at $22^\circ \pm 1^\circ \text{C}$. Twelve animals were tested in each salinity. After 12 hours, each animal was removed from the test solution, the contents of the mantle cavity drained into the test solution and the animal placed into 700 ml of fresh sea water of the same osmotic concentration. Using this transfer technique, animals were placed in fresh sea water every 12 hours for 84 hours. Sea water samples were removed from the beakers and assayed for ammonia nitrogen at the 12, 24, 36, 60 and 84 hour transfer times, following the phenolhypochlorite method as modified by Solorzano (1969). In this procedure, ammonia is reacted with phenol and hypochlorite at a high pH to produce indophenol, a blue-colored product. The indophenol concentration was determined spectrophotometrically (Spectronic 20; Bausch and Lomb) at 640 nm and converted to ammonia concentration by comparison with a standard curve. At the end of the experiment, the soft parts of each mussel were removed from the shell, freeze-dried and weighed to obtain a dry tissue weight.

Since preliminary experiments, as well as those of other investigators (Hammen, 1968; Emerson, 1969), indicated an influence of body weight on excretion rates within a treatment group, this effect was removed as follows: a pooled within-treatment regression line was calculated using data from the 84-hour treatment groups; the slope of this line was then used to adjust individual excretion rate values at all time periods to the grand mean animal weight. The following equation was used: $Y_{\text{adjusted}} = Y_{\text{measured}} - b$ (individual weight - grand mean weight), where Y is the ammonia excretion rate and b is the slope of the pooled within-treatment regression line. Adjusted means of treatment groups at each time period were then calculated.

For this experiment, and for all subsequent experiments unless otherwise specified, means of treatment groups at each time period were compared statistically using the Student's t -test. P -values less than 5% were accepted.

Ammonia production of isolated valves

An additional control experiment was done to determine the amount of ammonia production which could be attributed to nonmussel sources (*e.g.*, bacteria) remaining on the valves of the scrubbed mussels. Animals acclimated to full strength sea water were dissected from their scrubbed shells. The valves (five pairs per treatment group) were then treated as experimental and control animals in an experiment identical to the 84-hour time course study described above.

Ammonia concentration in hemolymph during acclimation

The concentration of ammonia in the blood of propped-open experimental and control mussels was measured 0, 4, 12, 24 and 60 hours following initial exposure of animals to test sea water in an experiment conducted as previously described. However, since animals had to be sacrificed to obtain blood samples, a different group of mussels (twelve per treatment group) was used to obtain blood ammonia values for each time period.

Each blood sample was obtained by removing one valve of a mussel, blotting the mantle and visceral mass dry with filter paper and slashing the mantle and adductor muscles with a scalpel blade. The anterior end of the animal was then placed into the mouth of a 50 ml centrifuge tube and both the animal and tube wrapped tightly with Parafilm to prevent evaporation. Hemolymph was allowed to drain from the cut tissue surfaces into the tube for 20–35 minutes at 4° C. The collected blood was then centrifuged at 3000 *g* for ten minutes to remove any contaminating tissue debris. Hemolymph collected by this technique is osmotically equivalent to hemolymph removed directly from the circulatory system by syringe (Pierce, 1970). Therefore, significant contamination of the blood from intracellular sources is unlikely. Blood samples were appropriately diluted with deionized water to a final volume of 5 ml and then analyzed for ammonia nitrogen as described above.

Free amino acid concentrations in hemolymph during acclimation

The concentrations of amino acids in the blood of propped-open experimental and control mussels were measured 0, 2, 4, 12, 24 and 60 hours following introduction of animals into test sea water. Test salinities and water change intervals were the same as in the previously described experiments. At the end of each time period blood samples were collected from six mussels in each salinity. Appropriate volumes of blood were diluted with glass distilled water to a final volume of 1 ml. Amino acids were extracted and proteins precipitated by adding 1 ml of 80% ethanol to each diluted blood sample. This mixture was brought to a boil in a water bath, allowed to extract below the boiling point for 30 minutes and then centrifuged at 20,000 *g* for 30 minutes. The supernatant solution was saved and the precipitate washed once with 2 ml of 40% ethanol and centrifuged again. The supernatant solutions from both centrifugations were pooled and lyophilized. The resulting residue was dissolved in an appropriate volume of sodium citrate buffer (pH 2.2) and analyzed for acidic and neutral amino acids with an amino acid analyzer (JEOL—Model JLC-6AH). Previous investigators have shown that acidic and neutral amino acids are the major constituents of the free amino acid pools of *Modiolus* blood and tissues and are also the only intracellular amino acids utilized for cell volume regulation during salinity stress (Pierce 1971b; Pierce and Greenberg, 1972, 1973).

The amino acid analysis of blood samples consistently revealed a peak with the same elution time as that of the urea contained in our amino acid standard mixture. Since urea excretion is uncommon among bivalves (Campbell and Bishop, 1970), an experiment was performed to determine whether or not this peak was actually urea. Urease (Sigma Chemical Co.) was dissolved in phosphate buffer

prepared according to the method of Welsh, Smith, and Kammer (1968). A blood sample was divided into four aliquots. A known amount of urea was added to one aliquot, urea plus urease was added to the second, urease alone was added to the third and the fourth aliquot was left unaltered. All four solutions were incubated for 30 minutes at 30° C. Ethanol extraction and amino acid analysis were then completed as described above.

Free amino acid concentrations in tissues during acclimation

The concentrations of amino acids in specific tissues were measured at various times following introduction of propped-open animals into test sea water. Ventricles were removed at the end of 4, 12 and 24 hour exposures. Mantle samples were taken at 4, 12, 24 and 60 hours. At each time period tissues were removed from 32 animals (16 experimental and 16 control), frozen on dry ice and lyophilized. The dry tissue pieces, in groups of four, were weighed and then homogenized and extracted in 80% ethanol as previously described. Concentrations of acidic and neutral amino acids were determined with the amino acid analyzer.

Natural acclimation of mussels to low salinity

A large group of mussels was placed into 50% sea water and allowed to acclimate to that salinity for at least five weeks without being propped open. This period of time is more than adequate to ensure complete osmotic acclimation of *M. demissus* to any non-lethal salinity (Pierce, 1970).

Ammonia excretion rates following acclimation

At the end of the acclimation period, 22 animals were scrubbed, propped open and placed into Millipore filtered 50% sea water for 24 hours. The mussels were then transferred into individual beakers of fresh 50% sea water. This water was changed after 12 hours and the concentration of ammonia collected during the 12 to 24 hour interval was determined. Similar measurements in full strength sea water were made on 20 animals acclimated to full strength sea water for five weeks. Analysis of covariance was employed to remove the effect of weight on excretion rates and to compare the means of the experimental and control groups.

Free amino acid concentrations in hemolymph following acclimation

At the end of the five week acclimation period, blood samples were removed from twelve mussels and analyzed for amino acids according to the methods presented above. Blood amino acid concentrations were also determined for twelve animals naturally acclimated to full strength sea water for five weeks.

RESULTS

Ammonia excretion rates during and following acclimation

In all the experiments described below, a significant correlation ($P < 0.01$) was found between ammonia excretion rate and dry tissue weight. Larger animals had smaller excretion rates irrespective of salinity (Fig. 1). To compensate for this

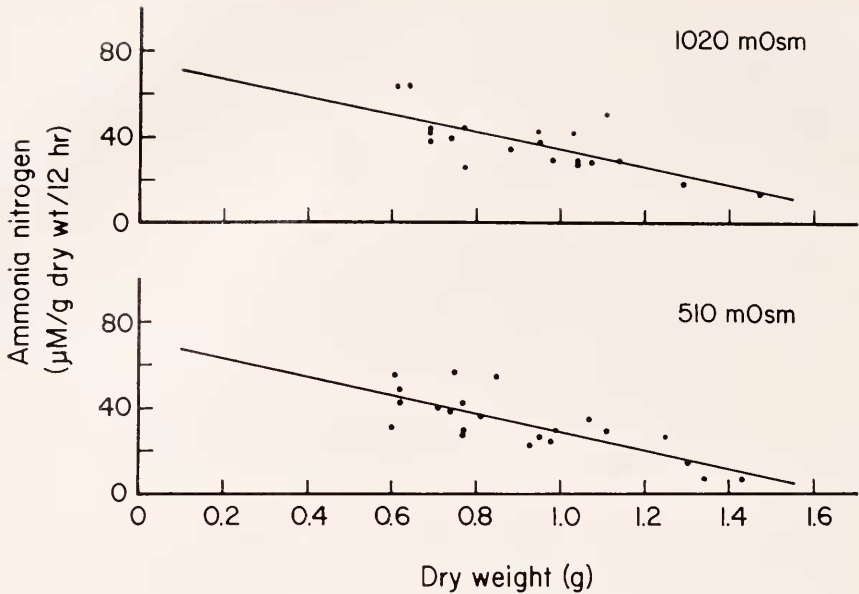


FIGURE 1. Linear regression analyses of ammonia excretion rate as a function of dry tissue weight for propped-open *M. demissus* in full strength sea water (1020 mOsm/kg H₂O) and 50% sea water (510 mOsm/kg H₂O). Each point represents one animal. Mussels were previously acclimated to their respective salinities for five weeks. Regression line formulae are: full strength sea water, $Y = -40.88x + 74.64$, $r = -0.719$; 50% sea water, $Y = -42.70x + 71.23$, $r = -0.777$.

weight effect, all measured ammonia excretion rates were statistically adjusted (see Methods) to the grand mean dry weight of the mussels used in each experiment. The data presented below are the adjusted values.

During low salinity acclimation, no significant difference was found between excretion rates of control and experimental animals during the first 12 hours of exposure to the test salinity (Fig. 2). However, during the 12 to 24 hour, 24 to 36 hour and 48 to 60 hour time periods, a significantly higher ammonia excretion rate occurred in the experimental animals ($P < 0.01$). The excretion rate of animals in low salinity reached a maximum of $51.7 \mu\text{M NH}_3\text{-N/g dry wt/12 hours}$ during the 12 to 24 hour collection period, leveled off during the 24 to 36 hour interval and then gradually began to decline toward control rates. However, the mean excretion rate of the experimental animals was still greater than that of the control animals during the 72 to 84 hour time period ($P < 0.05$).

The mean ammonia excretion rates (\pm standard errors) of mussels naturally acclimated for five weeks to full strength and 50% sea water were 37.2 ± 2.01 and $32.1 \pm 1.92 \mu\text{M NH}_3\text{-N/g dry wt 12 hours}$, respectively. The difference between the two excretion rates was not significant.

Concentrations of ammonia produced by isolated valves of *M. demissus* during the 84 hour time course of low salinity exposure are listed in Table I. The valves in 50% sea water produced more ammonia than those in full strength sea water at

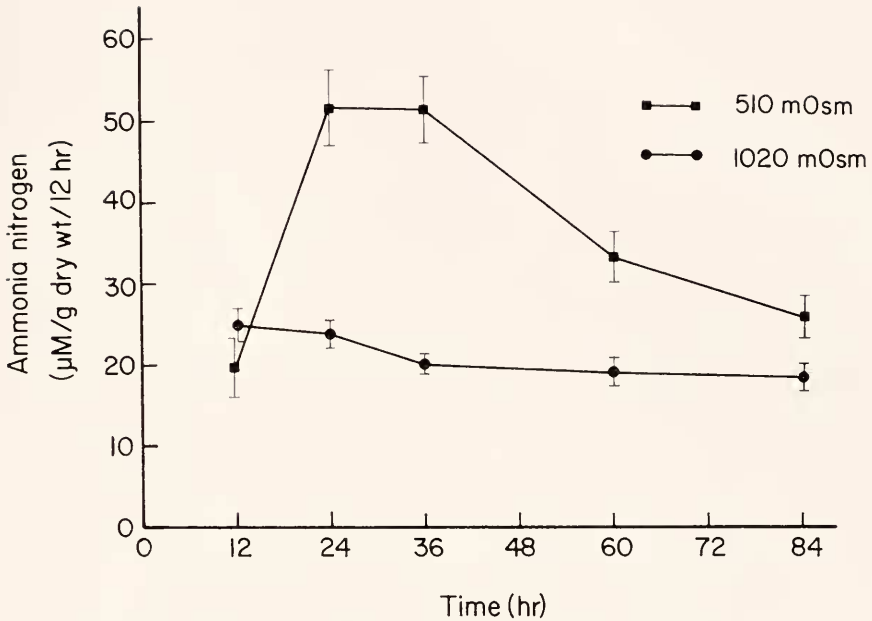


FIGURE 2. Time course of changes in ammonia excretion rates of propped-open *M. demissus* during low salinity acclimation. Mussels were previously acclimated to full strength sea water. Each point represents the mean excretion rate of twelve animals during the previous 12 hour interval. Error bars represent standard error.

all time intervals except the first one (0–12 hours). However, the quantities of ammonia produced by the isolated valves were in all cases less than the standard errors of whole animal excretion rates measured at the corresponding time intervals. Therefore, these nonmussel sources of ammonia were not a source of contamination.

Ammonia concentration in hemolymph during acclimation

Blood ammonia concentrations in control and experimental animals were not significantly different following both 4 and 12 hours exposure to test sea water

TABLE I

Ammonia production of isolated valves of M. demissus. Each value represents the mean ammonia production of five pairs of valves during the previous 12-hour interval.

Time (hour)	Ammonia production	
	1020 mOsm, kg H ₂ O	510 mOsm, kg H ₂ O
12	1.7 (±0.07)*	1.3 (±0.43)
24	1.2 (±0.07)	1.6 (±0.21)
36	1.1 (±0.07)	1.3 (±0.21)
60	0.9 (±0.07)	2.7 (±0.29)
84	1.0 (±0.07)	1.9 (±0.36)

* $\mu\text{M NH}_3\text{-N/12 hour}$ (±SE).

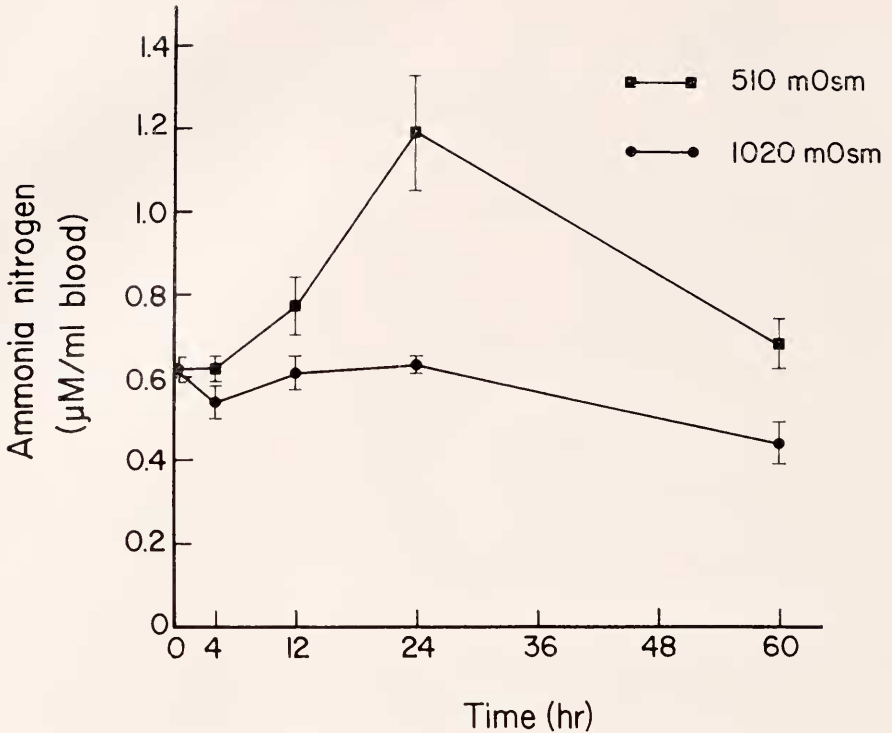


FIGURE 3. Time course of changes in hemolymph ammonia concentrations of propped-open *M. demissus* during low salinity acclimation. Mussels were previously acclimated to full strength sea water. Points are mean values from twelve animals. Error bars designate standard error.

(Fig. 3). After 24 hours, however, experimental animals had a significantly higher concentration of ammonia in the blood than did the control animals ($P < 0.01$), reaching a maximum concentration of $1.2 \mu\text{M NH}_3\text{-N/ml}$ blood. Analysis of 60-hour samples showed that ammonia concentrations in the experimental animals were returning toward control levels by this time but were still significantly higher ($P < 0.01$).

Free amino acid concentrations in hemolymph during and following acclimation

Urea was not found in the hemolymph of *M. demissus*. Amino acid analyses of unaltered blood, a mixture of blood and urease and a mixture of blood, urea and urease resulted in identical chromatograms of the compound eluting at the same time as urea in our standard amino acid mixture. Therefore, this compound is not urea and remains unidentified.

During acclimation the changes in total free amino acid concentration in the blood of experimental mussels (Fig. 4) followed a pattern similar to the changes in blood ammonia, except that the elevation of amino acid concentration preceded the rise in blood ammonia (Fig. 3). After both 4 and 12 hours exposure to test

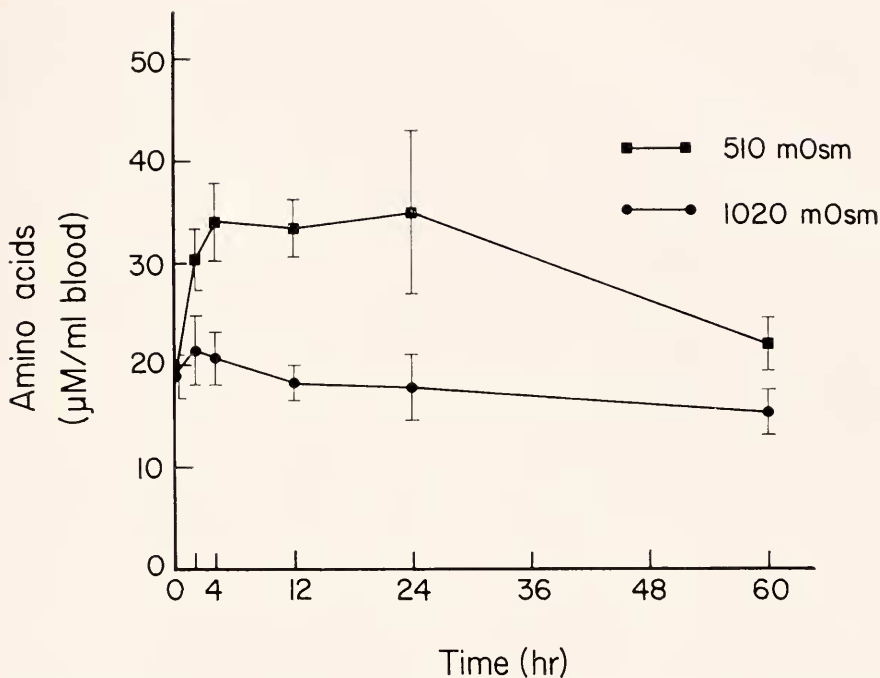


FIGURE 4. Time course of changes in total free amino acid concentrations in the hemolymph of propped-open *M. demissus* during low salinity acclimation. Mussels were previously acclimated to full strength sea water. Points are mean values from six animals. Error bars represent standard error.

salinities, the total free amino acid concentration of the blood was significantly higher in the experimental mussels (4 hours, $P < 0.05$; 12 hours, $P < 0.01$). At the 24 hour sampling period the mean free amino acid concentration in the blood of experimental animals was still much higher than the control mean concentration. However, the variation in concentrations within the experimental group was large enough so that no statistical difference could be found between the two groups of mussels. After 60 hours the total free amino acid concentration in blood of the animals in low salinity had declined toward control values and was not significantly different.

Following complete acclimation to low salinity, mussels have total blood amino acid concentrations much lower than those of animals acclimated to full strength sea water. The mean total free amino acid concentrations (\pm standard errors) in blood of animals acclimated for five weeks to full strength and 50% sea water were 18.9 ± 2.15 and 10.6 ± 1.08 μM amino acids/ml blood, respectively.

Free amino acid concentrations in tissues during acclimation

The total free amino acid pool in ventricles of mussels acclimating to 50% sea water decreased steadily with time (Fig. 5). Mean values of total amino acids

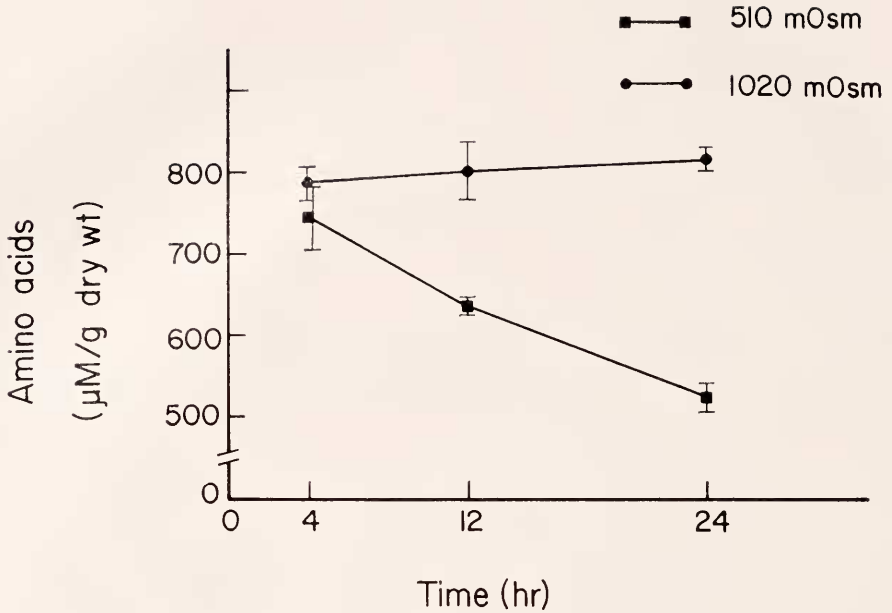


FIGURE 5. Time course of changes in total intracellular free amino acid concentrations in ventricles removed from *M. demissus* during low salinity acclimation. Mussels were previously acclimated to full strength sea water. Each point represents the mean of four tissue homogenates, each containing four ventricles. Error bars designate standard error.

in the ventricles of experimental animals were lower than in ventricles of control animals at all times investigated. These differences were significant ($P < 0.01$) at the 12 and 24 hour sampling periods.

Concentrations of total free amino acids in mantle tissue of experimental and control animals at various times during low salinity acclimation are presented in Table II. There was no significant difference between the control and experimental amino acid pools at any time period examined.

TABLE II

Changes in total intracellular free amino acid pool in mantle tissue of propped-open M. demissus during low salinity acclimation. Each value represents the mean of four tissue homogenates each containing mantle tissue from four mussels.

Time (hour)	Amino acid concentration	
	1020 mOsm/kg H ₂ O	510 mOsm/kg H ₂ O
4	675.4 (± 18.50)*	712.6 (± 18.23)
12	662.3 (± 33.88)	684.9 (± 31.77)
24	684.4 (± 34.68)	706.9 (± 29.27)
60	718.3 (± 45.76)	705.6 (± 30.51)

* $\mu\text{M/g}$ dry tissue wt. ($\pm\text{SE}$).

DISCUSSION

During volume regulation of isolated ventricles of *M. demissus* to low salinity, free amino acids rapidly efflux from individual cells along with osmotically obligated water (Pierce and Greenberg, 1972). The data presented here show clearly that this same mechanism operates in the whole animal during low salinity acclimation. Namely, in response to lowered external osmotic pressure, cell swelling is controlled by the rapid release of free amino acids from the intracellular pool into the hemolymph. The time course of the elevation in blood amino acid concentration reported here corresponds closely to the rate of change in hemolymph osmolality in propped-open *Mytilus edulis*, a closely related species, over a similar salinity range (Costa, 1976). The amino acids released into the hemolymph are not excreted from the whole animal. Instead, several hours after the rise in hemolymph amino acid concentration, blood ammonia levels increase coincident with increased external ammonia excretion rates. As acclimation proceeds, the amino acid concentration of the blood decreases back toward control levels, followed by a decrease in blood ammonia. Finally, external ammonia excretion rates return to control levels. Therefore, it appears that the amino acids are being degraded, rather than excreted, some time after leaving the cell as osmotic solute.

The exact time relationship between increased blood ammonia concentrations and elevated external ammonia excretion rates is not clear. Blood ammonia concentrations first appear elevated at 24 hours following the salinity change, which is both considerably after the initial amino acid release and in the midst of the peak of external ammonia excretion rates (12–36 hours). We did not measure either blood ammonia or external ammonia at intermediate time intervals between 12 and 24 hours. However, since excess ammonia usually diffuses rapidly out of the tissues of aquatic animals these two phenomena may coincide.

The ammonia excretion rates of mussels acclimated to low salinity are the same as those of animals acclimated to full strength sea water. Moreover, the total free amino acid pool of the blood of the low salinity mussels is lower than that of animals in full strength sea water. Therefore, the high levels of hemolymph amino acids and the elevated ammonia excretion rates observed during acclimation are transitory and do not persist after acclimation to low salinity is complete.

In spite of the transient increase in ammonia excretion rates by *M. demissus* during low salinity acclimation, the mechanism of intracellular free amino acid regulation is unlike that which has been proposed for Crustacea (Florkin and Schoffeniels, 1965, 1969; see introduction). First, it is obvious that blood free amino acid levels in acclimating mussels become elevated before blood ammonia concentrations increase. Therefore, in the whole animal amino acids are released intact from cells into the blood, as Pierce and Greenberg (1972) have demonstrated with isolated tissue, rather than being degraded intracellularly. In fact, similar changes in blood amino acid concentrations have also been observed in crustaceans (Vincent-Marique and Gilles, 1970; Haberfield, 1971; Gerard and Gilles, 1972), as well as in several polychaete annelids (Clark, 1968) and *Mytilus edulis*, another mussel (Potts, 1958; Bricteux-Grégoire, Duchâteau-Bosson, Jeuniaux, and Florkin, 1964). Second, since peak concentrations of ammonia both in the blood and outside the animal follow the elevation of free amino acid concentrations in the blood, the increased production is most likely due to degradation of amino acids by a

particular organ of the body following the volume regulation response. A similar mechanism has been suggested to reconcile proline release from cells of the crab *Eriocheir sinensis* and the lack of external proline excretion during low salinity stress (Vincent-Marique and Gilles, 1970).

The free amino acid pool of the mantle tissue is reduced in *M. demissus* completely acclimated to lower salinity (Pierce, 1971b). However, while the free amino acid concentration of the ventricle is already decreased 12 hours after the animal is exposed to low salinity, the pool in the mantle has not begun to decrease, even after 60 hours. The mantle may simply have a much lower permeability to water and ions than other tissues. On the other hand, the mantle may well be a site of accumulation and degradation of amino acids released by other tissues during salinity stress. Read (1962) and Awapara and Campbell (1964) have shown the presence of amino-transferase activity in the mantle tissue of various bivalves. Furthermore, the mantle is highly vascularized and, since it presents a large surface area in direct contact with the external environment, a rapid release of large quantities of toxic ammonia could be achieved as soon as the ammonia is produced. Confirmation of this hypothesis awaits considerable experimental evidence.

Finally, the ammonia excretion rates determined in this study are 10 to 15 times greater than those determined for *M. demissus* in full strength sea water by previous investigators (Lum and Hammen, 1964). A number of factors may have contributed to the large discrepancy between excretion rates measured in the two studies. First of all, Lum and Hammen did not prop open the shells of the mussels nor, apparently, did they drain the mantle cavity before water samples were taken for ammonia analysis. Second, Lum and Hammen placed six to ten animals together in 50 ml of water and left them in that same water for 24 hours prior to analyzing the water for ammonia. Needham (1957) has demonstrated depression of nitrogen output when animals are grouped in a limited volume of medium. Moreover, ammonia accumulation in the external environment inhibits further production of ammonia by organisms (Needham, 1957; Lum and Hammen, 1964). Finally, in calculating excretion rates Lum and Hammen did not use measured tissue weights. Rather the tissue weights were calculated from the whole animal weight (including shell), using a correction factor determined in a previous experiment (Hammen, Hanlon, and Lum, 1962). This correction factor assumes that the dry tissue weight equals 35% of the wet weight (tissue and fluid) of the whole animal. This percentage is 2 to 3.7 times higher than measured values determined by other investigators (Martin, Harrison, Huston, and Stewart, 1958; Pierce, 1971a). Thus, the estimated weights used by Lum and Hammen (1964) may have been larger than the true animal dry weight and therefore the ammonia excretion rates calculated would be smaller than the actual excretion rate.

SUMMARY

1. Free amino acid concentrations in the hemolymph increase rapidly in company with a decrease in the tissue free amino acid pool of propped-open *M. demissus* exposed to low salinity.

2. As acclimation proceeds, blood amino acid concentrations level off and an increase in both hemolymph ammonia concentration and external ammonia excretion occurs.

3. Finally, hemolymph amino acid concentrations decrease toward control levels, followed by hemolymph ammonia concentration and external ammonia excretion rates.

4. Following complete acclimation of mussels to high or low salinity, ammonia excretion rates are the same. However, hemolymph free amino acid concentrations are lower in animals adapted to low salinity.

5. These results show that during acclimation of mussels to low salinity, free amino acids are released intact from the cells into the hemolymph. The subsequent transitory increase in hemolymph ammonia concentrations and external ammonia excretion rates is probably due to degradation of the effluxed amino acids by some specific organ of the body.

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